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Study of antifungal susceptibility pattern of various *Candida species* with reference to conventional and automated methods in a tertiary care hospital

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Abstract: *Introduction:* The incidence of *Candida* infections is on the rise with the increase in number of immuno-compromised patients due to excessive use of immunosuppressive drugs as well as the use of medical and surgical interventions. *Materials and Methods:* A cross-sectional observational study was conducted in a tertiary care hospital from February 2021- August 2022. All *Candida* isolates from various clinical samples during the study period were processed for identification and susceptibility testing by both Automated & Conventional methods with E-strip. Data analysed by using Microsoft excel spreadsheet and then analyzed by SPSS and Graph PadPrism version 5. *Result:* Total *Candida* isolates were 100, among them near about half (43%) of the isolates were obtained from critical care unit followed by thoracic unit (17%), Ward (16%), trauma centre (15%), high dependency (7%) and 2% from outdoor. Maximum number of isolates came from central line catheter 43, followed by blood 19. other samples from where candida were isolated are urine 13, endotracheal aspirate 9, Pleural fluid 7, Bronchoalveolar lavage 3, Cerebrospinal Fluid 3 and Wound 3. Isolated pathogens were *Candida albicans*. *Candida tropicalis*, *Candida parapsilosis*, *Candida glabrata*, *Candida auris*, *Candida guilliermondii* , *Candida haemulonii*, *Candida ciferrii*, *Candida famata*, *Candida duobushaemulonii*. The association between isolated Candida and from which samples they are isolated were statistically significant with 'P' value <0.0001 and Chi-square value: 284.4447. *Conclusion:* The increasing incidence of HIV infections, increasing incidence of organ transplantation, wide spread use of antibiotics and use of immunosuppressive agents are the important causative factors responsible for the increasing incidence of *Candida* infections. Furthermore, due to increasing resistance to various antifungal agents *Candida* has assumed greater clinical importance. This observation of the present study is in consonance with the changing trend seen both in India and across the world.

Keywords: Candida, AFST, Fluconazole, Non-Albicans, Infection

Introduction

Hospital acquired infection with multi-drug resistance microorganism is increasing day by day. Other than multi-drug resistance bacterial infection, Candida infections often associated with high morbidity and mortality have increased remarkably during the couple of decades [1]. The incidence of Candida infections is on the rise with the increase in number of immuno-compromised patients due to excessive use of immunosuppressive drugs as well as the use of medical and surgical interventions.However, large proportions of these infections are preventable by effective infection prevention and control measures.

Candida species, by virtue of being the component of normal flora of human beings, mostly causes endogenous infection due to alteration of balance of normal microbial flora of human body or by lowering host resistance, the causes of which are increasing trends of indiscriminate use of antibacterial antibiotics, increasing HIV cases and increasing incidence of organ transplantation. Although *Candida albicans* is the most prevalent species, an epidemiological shift in Candida pathogens

has been recently noted by the increasing number of infections caused by non-albicans candida species (NAC) [2-3].

The immergence of NAC isolates and the increasing resistance among NAC isolates to multiple antifungal drugs is a matter of concern to initiate empirical antifungal therapy. Species level identification is clinically important due to the fact that they differ in virulence and antifungal susceptibility. The increased species diversity and incidence of infections have resulted in the need for an accurate and Rapid identification of candida species and their antifungal susceptibility testing (AFST) pattern to early initiation of treatment and hence rapid management before complications and for the prevention of emergence of drug resistance [2, 4].

Study aims to find out the occurrence of various candida infection and the AFST of the isolates for early identification of isolates and initiation of definitive treatment. The comparison of both automated and conventional methods for identification and susceptibility pattern detection is important because in resource constrained laboratory automated system is not available.

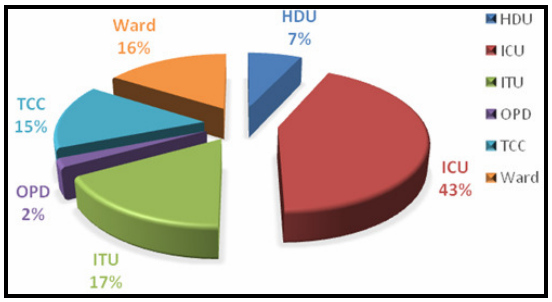
Material and Methods

This cross-sectional observational study was conducted in a tertiary care hospital of Kolkata from February 2021- August 2022. All candida species isolated from various clinical samples during the study period were processed by both automated (VITEK-2 Compact system) and conventional methods with E-strip. Data analysis was done by using Microsoft excel spreadsheet and then analyzed by SPSS (version 27.0; SPSS Inc., Chicago, IL, USA) and Graph PadPrism version 5.

Results

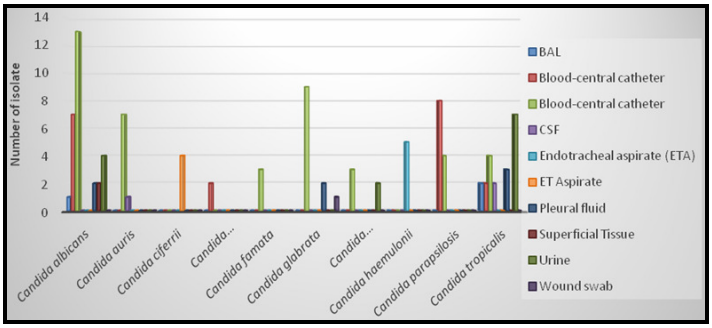
In our study, total candida isolates were 100, among them near about half (43%) of the isolates were obtain form ICU patient followed by ITU 17%, Ward 16% TCC 15% HDU 7% and 2% from OPD (Fig-1). Maximum number of candida isolated from central line catheter 43, followed by blood 19. other samples from where candida were isolated are urine 13, Endotracheal aspirate (ETA) 9, Pleural fluid 7, BAL 3, CSF 3, Wound 3.

Fig-1: Distribution of samples (n=100)



In our study 71(71%) isolates were NAC where only 29 (29.0%) isolates were *Candida albicans*. Among NAC isolates, most common isolate was *Candida tropicalis* 20 followed by *Candida parapsilosis* 12, *Candida glabrata* 12, *Candida auris* 8 , *Candida guilliermondii* 5, *Candida haemulonii* 5, *Candida ciferrii* 4,*Candida famata* 3, *Candida duobushaemulonii* 2. Maximum *Candida albicans* were isolated from central line. *Candida auris* mainly isolated from central line and BAL fluid where *Candida tropicalis* were isolated mainly from urine. The association between isolated *Candida* and from which samples they are isolated were statistically significant with p value <0.0001 and Chi-square value: 284.4447 (Fig-2).

Fig-2: Association between *Candida spp* and type of samples from where they have been isolated



In this study we use both conventional and automated methods for identification and detection of antifungal susceptibility pattern of candida species. Regarding identification of candida species, there was 100% concordance in both methods.

Regarding detection of antifungal susceptibility pattern of candida species by both methods, we found that candida species were more susceptible to micafungin (94%) in both method followed by voriconazole (87% by VITEK-2, 88% by E-strip) and anidulofungin (78%) by both methods (Table -1). Highest resistant was showed against fluconazole (28%) by both methods. The highest rate of fluconazole resistance was observed in *C. cifferii* (4 isolates out of 4) followed by *C. auris* (7 isolates out of 8). Some of the non-albicans candida are intrinsically resistant to fluconazole due to enzyme modification like *C.glabrata*. This high level of resistance of fluconazole might be due to overuse of antifungal agents and also their empirical therapy in our scenario. Most sensitive candida isolates were *Candia famata* and *Candida parapsilosis* showing cent percent susceptibility towards all five antifungal in both the methods. *Candida tropicalis* shows excellent susceptibility towards all antifungal except one isolate (showing resistance to fluconazole). *Candida auris* found to be most resistant candida species, in this study, followed by *Candida albicans*.

Discordance in antifungal susceptibility has been observed in this present study between conventional and automated methods as shown in Table-2.

Table-1: Comparison of resistance pattern between two methods in terms of number of isolates

	Anidulaf-ungin		Caspofungin				Fluconazole			Micafungin				Voriconazole	
	Sensitive	Resistance	Sensitive	Intermediate	Resistance	Not determined	Sensitive	Intermediate	Resistance	Sensitive	Intermediate	Resistance	Sensitive	Intermediate	Resistance
Automated	78	22	72	1	20	7	67	5	28	94	1	5	87	2	11
Conventional	78	22	74	0	21	5	67	5	28	94	1	5	88	0	12

Table-2: Discordance of resistance pattern between two methods among candida species

Isolates		Voriconazole			Caspofungin		
		S	I	R	S	I	R
C. albicans	Automated	23	00	06	21	00	02
	Conventional	23	00	06	26	00	03
C.glabrata	Automated	10	01	01	04	01	07
	Conventional	09	00	03	05	00	07
C.auris	Automated	05	01	02	02	00	06
	Conventional	05	00	03	02	00	06

Discussion

Candida species are usually normal commensal of our skin and mucus membrane but they can produce both invasive and non invasive infections specially when host immunity decrease. The increasing incidence of HIV infections, increasing incidence of organ transplantation, wide spread use of antibiotics and use of immunosuppressive agents are the important causative factors responsible for the increasing incidence of Candida infections. Furthermore, increasing resistance to various antifungal agents Candida has assumed greater clinical importance.

In our study maximum number of *Candida ssp*. were isolated from central line catheter 43, followed by blood 19 and urine 13, where as a study conducted in Pune showed maximum number of *Candida spp* were isolated from urine followed by blood [5]. Of the 100 Candida isolates, 29% were *C. albicans* and 71% were non albicans among which *C. tropicalis* (20%), *C. parapsilosis* (12%), *C. glabrata* (12%) have been identified respectively. So, the prevalence of NAC was more when compares to *C. albicans*. This observation is in consonance with the changing trend seen both in India and across the world [6-10], whereas Wingard et al (1995) and Sajjan et al found *Candida albicans* as the predominant [11-12].

Among the NAC species, *C. tropicalis* (20%) was the most common isolates followed by *C. parapsilosis* (12%) and *C. glabrata* (14%). Jayalakshmi et al. also showed that *C. tropicalis* (26.6%) was prevalent among the NAC species [13]. Similar result has been observed in different studies from different countries of Europe [14-15]. For identification of *Candida spp* we used both conventional and VITEK 2 compact system. We found 100% concordance between two methods. Where as in another Indian study showed 6 out of 50 isolates were misidentified a *C.famata* (*C.glabrata*- 02, *C.albicans*- 02, *C.kefyr*- 01,*C.guilliermondii*- 01) by VITEK 2 [5].

In our study we found that candida species were more susceptible to micafungin (94%) followed by voriconazole and highest resistant was showed against fluconazole (28%). Similarly, another study in Bangalore showed 100% susceptibility to voriconazole, whereas highest degree of resistance was observed for fluconazole [16].

We found that maximum fluconazole resistant was seen in *C. cifferii* followed by *C. auris* but opposite result found by Barchiesi F et al (2000) [17].

Among 29 *Canida albicans* isolates, 27(93%) showed sensitivity to both fluconazole and caspofungin. *Candida tropicalis* showed 95% and 100% sensitivity to fluconazole and caspofungin respectively. Quite similar findings shown by Talukdar A et al where 40 *C. albicans* isolates were sensitive to caspofungin 40(100%) and 34 (85%) sensitive to fluconazole [18].

In our study we found 87 candida isolates were susceptible to voricozazole by VITEK and 88 isolates susceptible by E-strip, but there is no discrepancy in case of fluconazole susceptibility by both methods. In contradictory another study they showed 4% (02 isolates) as resistant to fluconazole by VITEK and by E-test method, 48% (24 isolates) of isolates were found to be resistant to fluconazole [5].

Conclusion

Prevalence of non-albicans Candida infections was more as compared to *C. albicans*. Among the non-albicans Candida, *C. Tropicalis* was the most prevalent isolate. In our study we showed that both automated and conventional methods for identification and AFST of candida species were comparable.VITEK-2 is the optimum option whereever available for its rapidity, but conventional methods with E-strip are as reliable as the automated method and good option in resource limited laboratories.

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